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GENERALIZED MODELING FOR THERMAL INACTIVATION OF SALMONELLA IN MEAT AND ENHANCEMENT OF THE AMI PROCESS LETHALITY SPREADSHEET

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Introduction

In order to ensure the microbial safety of ready-to-eat (RTE) meat and poultry products, the U.S. Department of Agriculture (USDA) has required a 6.5-Log reduction in *Salmonella* spp. for cooked beef, RTE roast beef, and cooked corned beef products, and a 7-Log reduction in certain fully and partially cooked poultry products (USDA 1999). Additionally, USDA has proposed to extend the performance standards to all RTE meat products (USDA 2001). However, the ability of the industry to produce reliable and uniform thermal process validations on a broad scale has not been tested.

The existing tools for calculating process lethality do not account for all of the variables that might affect pathogen survival, and the acceptability of these tools for regulatory compliance has been recently questioned (USDA 2002). For example, the current version of the widely used American Meat Institute process lethality spreadsheet (AMI-PLS) calculates the F-value of a process, given time-temperature data, a reference temperature, and a z value. The user then must calculate log reductions, given a reference D value. Additionally, the AMI-PLS does not account for product characteristics, and therefore the user must know that the D and z values are valid for the product of interest. Unfortunately, a large percentage of the AMI-PLS users likely input the example D and z values provided in the spreadsheet, without knowledge of their validity for their specific products.

In general, temperature, fat content, and moisture content are important factors for thermal inactivation of *Salmonella*. Juneja (2001) conducted research on thermal inactivation of a *Salmonella* cocktail (Thompson, Typhimurium, Heidelberg, Enteritidis, Montevideo, Copenhagen, and Hadar) in different kinds of meat products, such as ground chicken, ground turkey, and ground beef. The general D-values at 58, 60, 62.5, and 65°C were around 7, 5, 1.5, and 0.6 min, respectively (depending on the product types). Murphy (2003) worked with a *Salmonella* cocktail in ground chicken breast and obtained D-values at 56, 60, 62, and 63°C of 3.2, 0.6, 0.31, and 0.18 min, respectively. Humphrey (1997) tested *Salmonella* Typhimurium DT 104 in ground beef and reported D-values at 58 and 60°C of 2.62 and 1.35 min, respectively. Table 1 (poultry) and Table 2 (beef) show the D-values for thermal inactivation of *Salmonella*, depending on the fat content, moisture content, and temperature.

Overall, the hypothesis of this project was that the D-value of *Salmonella* in meat products could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content.

Objectives

The specific objectives were: 1) To develop and validate a complete secondary model for logD as a function of temperature, fat content, and moisture content, with a 95% confidence limit as reference, and 2) To improve the AMI-PLS by integrating the new logD model and adding a user-friendly front-end.

Methodology

1. Data

Data Source Previous work (before 2004) was evaluated in the general area related to the project objective - product factors affecting thermal resistance of *Salmonella* in different kinds of meat (poultry, beef and pork). Most of related studies were identified through Combase, journal indexes, and in-house research data.

Data Selection Data were selected only when they met the following requirements: 1) temperature of 50-70°C; 2) experimental methods stated clearly; 3) fat content and moisture content analyzed; 4) D-value reported or could be calculated from the data; 5) good correlation (R^2>0.85) for logN and time in the data. After this screening, data from 18 different studies were selected for further analysis.

2. Model

Based on the temperature, fat content, and moisture content, the complete secondary model was selected by using back elimination (α =0.05). The variables were Fat, Moisture, and Temperature; the dependent variable was logD. The model was

$$LogD = \alpha + \beta_{11}T + \beta_{12}F + \beta_{13}M + \beta_{21}TF + \beta_{22}TM + \beta_{23}FM + \beta_{31}T^2 + \beta_{32}F^2 + \beta_{33}M^2 + \varepsilon$$

where D is the D value (min); T is temperature (°C); F is the fat content (%); and M is the moisture content (%).

3. AMI Spreadsheet Improvement

Currently, a user of the AMI-PLS must provide time-temperature data, along with D and z values at a reference temperature; however, the spreadsheet does not directly calculate log reduction for a given process. Our modification of the AMI-PLS includes: 1) development of a user-friendly front-end, via Visual Basic, for input of product attributes; 2) integration of the new generalized model for the D value; and 3) direct calculation of log reduction, and the confidence interval for those calculations. The front-end asks the user for the product species, product type, and product composition (fat, moisture, and pH). After selecting the product species and product type and inputting product compositions, clicking the submit button calculates the parameters for the logD model, which are imported directly into the spreadsheet. According to the data input, the spreadsheet reports the reference D-value at 62.8°C (±95% confidence interval), and the accumulated log reduction at each processing time.

Results & Discussion

Data Analysis *Salmonella* Senftenberg is the most thermally resistant species among all the species in *Salmonella*, but it is seldom the source of outbreaks related to meat and poultry. Therefore, data sets that included *Salmonella* Senftenberg were ignored in this study. Also, because the research methods were different from one laboratory to the other, and the range of D-value and z-value were large, some outlier data (where predicted minus actual logD>0.3 log[min]) were excluded, due to the need for more data to verify those values. The data used for the model are listed on Table 1 (poultry, 5 different studies from 4 researchers, 58 data sets) and Table 2 (beef, 3 different studies from 3 researchers, 28 data sets). The ranges of temperature, fat content, and moisture content for poultry were 55 - 65°C, 1 - 14.2%, and 67 - 75% respectively; the ranges of temperature, fat content, and moisture for beef were 55 - 65°C, 4.8 - 24%, and 57 - 72.4% respectively.

Model Selection The final model for poultry was:

$$\log D = -92.589 + 0.750F + 1.633M + 1.240T - 0.0035F^2 - 0.0101M^2 - 0.01T^2 - 0.0099FM + 0.0024MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 1. The root mean squared error (RMSE) was 0.08518 log(min). The final model for beef was

$$\log D = -237.59 + 2.307F + 3.309M + 4.086T - 0.0048F^2 - 0.0375FT - 0.0569MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 2. The RMSE was 0.1365 log(min).

AMI Spreadsheet Improvement The user-friendly front-end (Figure 3) includes the product species selection, product type selection, and product parameters input. After selecting the product species and product type and inputting product parameters, clicking the submit button leads the user to the spreadsheet (Figure 4). The data currently included in the model encompass only beef and poultry, no variation in product structure (e.g., whole vs. ground samples), and no meaningful variation in pH. Therefore, other species and product structure are currently non-active choices in the input box, and the pH input is merely returned in the output worksheet.

Conclusions

According to the data analysis and modeling, the D-value could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content. However, further research is needed to validate the model with independent results for more accurate prediction. Currently, the confidence intervals (CI) for the D-value are still quite wide. Therefore, more data sets are needed to narrow the CI and improve model accuracy, and the enhanced version of the AMI-PLS described here is not yet ready for distribution. Additionally, we are working on incorporating data for pork and different product structure (ground or whole muscle) into an expanded model set, which will enhance the AMI-PLS functionality.

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Tables and Figures

Table 1. List of product parameters and D-values for thermal inactivation of *Salmonella* in poultry.

Log D	Fat	Moisture	Temp	D value	Log D	Fat	Moisture	Temp	D value
0.875061	1	72	58	7.5	0.897627	1.1	72.3	55	7.90
0.658965	1	72	60	4.56	1.026125	1.1	72.3	55	10.62
0.184691	1	72	62.5	1.53	1.118595	1.1	76.3	55	13.14
-0.22915	1	72	65	0.59	0.895975	13.02	64.5	55	7.87
0.887054	7	72	58	7.71	0.913814	13.02	66.5	55	8.20
0.693727	7	72	60	4.94	1.039811	13.02	66.5	55	10.96
0.267172	7	72	62.5	1.85	0.994757	13.02	68.5	55	9.88
-0.25964	7	72	65	0.55	0.975891	1.1	72.3	55	9.46
0.839478	10	67	58	6.91	1.168203	1.1	71.5	55	14.73
0.710117	10	67	60	5.13	1.151982	1.1	71.9	55	14.19
0.161368	10	67	62.5	1.45	1.067443	1.1	71.4	55	11.68
-0.24413	10	67	65	0.57	0.993877	1.1	72	55	9.86
0.869818	12	68	58	7.41	1.079543	1.1	72.5	55	12.01
0.7348	12	68	60	5.43	1.016616	1.1	72.4	55	10.39
0.25042	12	68	62.5	1.78	1.089552	1.1	71.9	55	12.29
-0.22915	12	68	65	0.59	1.034227	13.02	68.5	55	10.82
0.870404	8.85	70.2	58	7.42	-0.29243	6.3	72	65	0.51
0.683047	8.85	70.2	60	4.82	0.931458	9	68	58	8.54
0.178977	8.85	70.2	62.5	1.51	0.732394	9	68	60	5.4
-0.09691	8.85	70.2	65	0.8	0.064458	9	68	62.5	1.16
0.665581	14.2	73	57	4.63	-0.27572	9	68	65	0.53
0.130334	14.2	73	60	1.35	0.956168	12	69	58	9.04
0.868056	2	75	58	7.38	0.740363	12	69	60	5.5
0.683947	2	75	60	4.83	0.113943	12	69	62.5	1.3
0.056905	2	75	62.5	1.14	-0.30103	12	69	65	0.5
-0.38722	2	75	65	0.41	0.850033	8.45	71.75	58	7.08
0.865104	6.3	72	58	7.33	0.716003	8.45	71.75	60	5.2
0.670246	6.3	72	60	4.68	0.133539	8.45	71.75	62.5	1.36
0.064458	6.3	72	62.5	1.16	-0.22915	8.45	71.75	65	0.59

Table 2. List of product parameters and D-values for thermal inactivation of *Salmonella* in beef.

Log D	Fat	Moisture	Temp	D value
0.507856	7	71	58	3.22
0.390935	12	65	58	2.46
0.396199	18	62	58	2.49
0.206826	24	57	58	1.61
0.956649	4.8	72.4	55	9.05
0.354108	4.8	72.4	58	2.26
-0.24413	4.8	72.4	61	0.57
-0.82391	4.8	72.4	64	0.15
1.023252	4.8	72.4	55	10.55
0.332438	4.8	72.4	58	2.15
-0.38722	4.8	72.4	61	0.41
-1.1549	4.8	72.4	64	0.07
1.01157	4.8	72.4	55	10.27
0.313867	4.8	72.4	58	2.06
-0.36653	4.8	72.4	61	0.43
-0.85387	4.8	72.4	64	0.14
1.342028	19.1	63.4	55	21.98
0.419956	19.1	63.4	58	2.63
-0.18709	19.1	63.4	61	0.65
-0.79588	19.1	63.4	64	0.16
1.270912	19.1	63.4	55	18.66
0.5302	19.1	63.4	58	3.39
-0.24413	19.1	63.4	61	0.57
-0.69897	19.1	63.4	63	0.2
0.937016	12.45	65.5	58	8.65
0.738781	12.45	65.5	60	5.48
0.176091	12.45	65.5	62.5	1.5
-0.17393	12.45	65.5	65	0.67

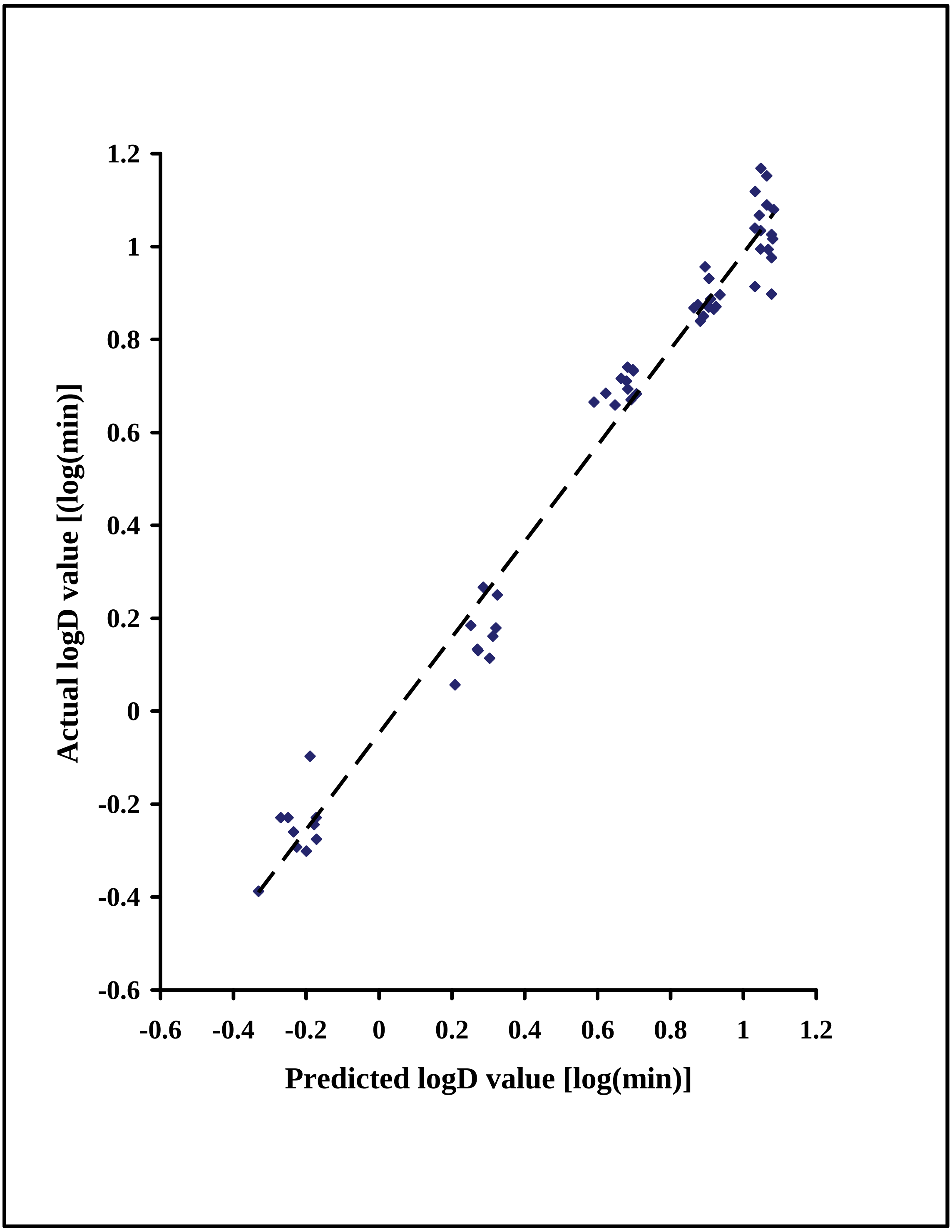

Figure 1. Observed vs. predicted log D from generalized model for thermal inactivation of *Salmonella* in poultry.

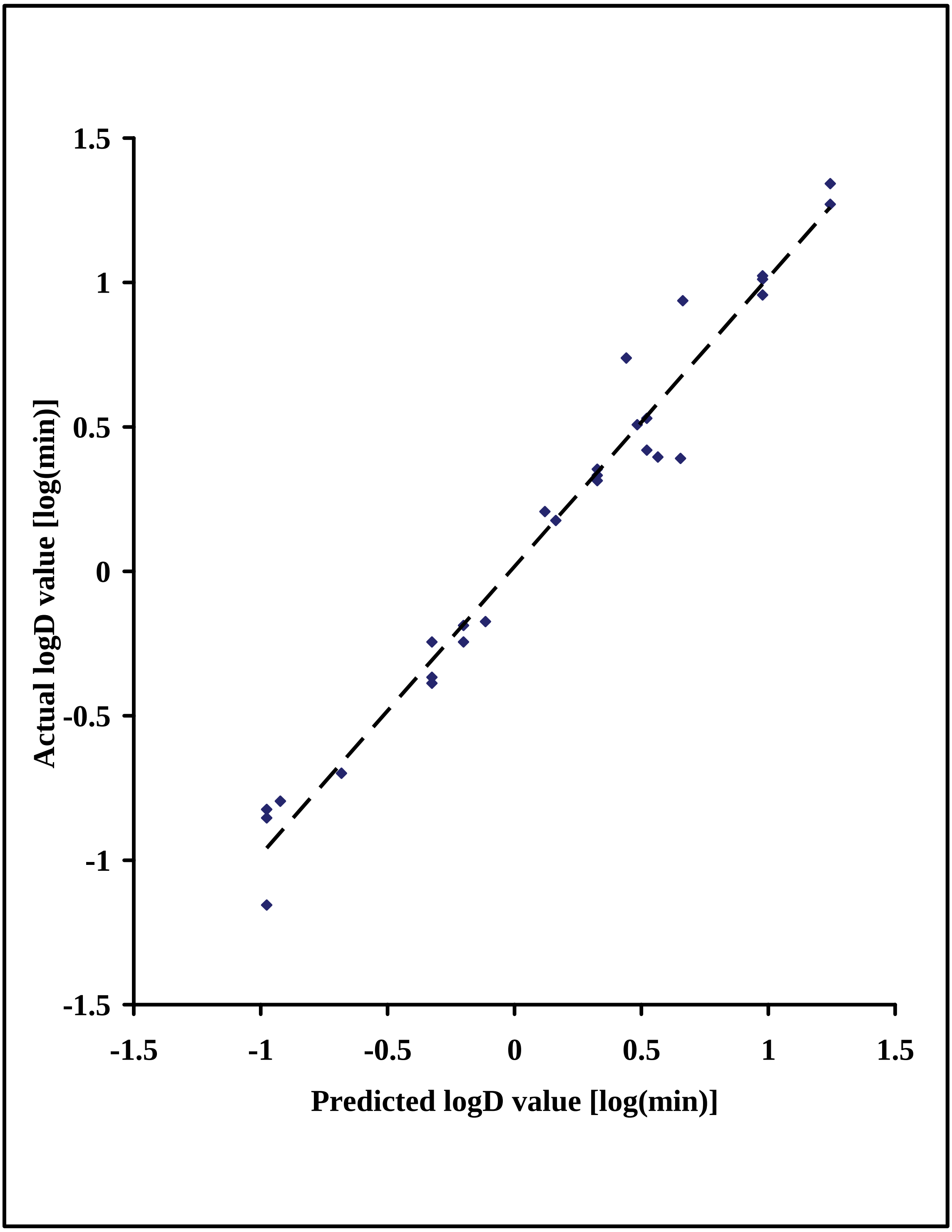

Figure 2. Observed vs. predicted log D from generalized model for thermal inactivation of Salmonella in beef.

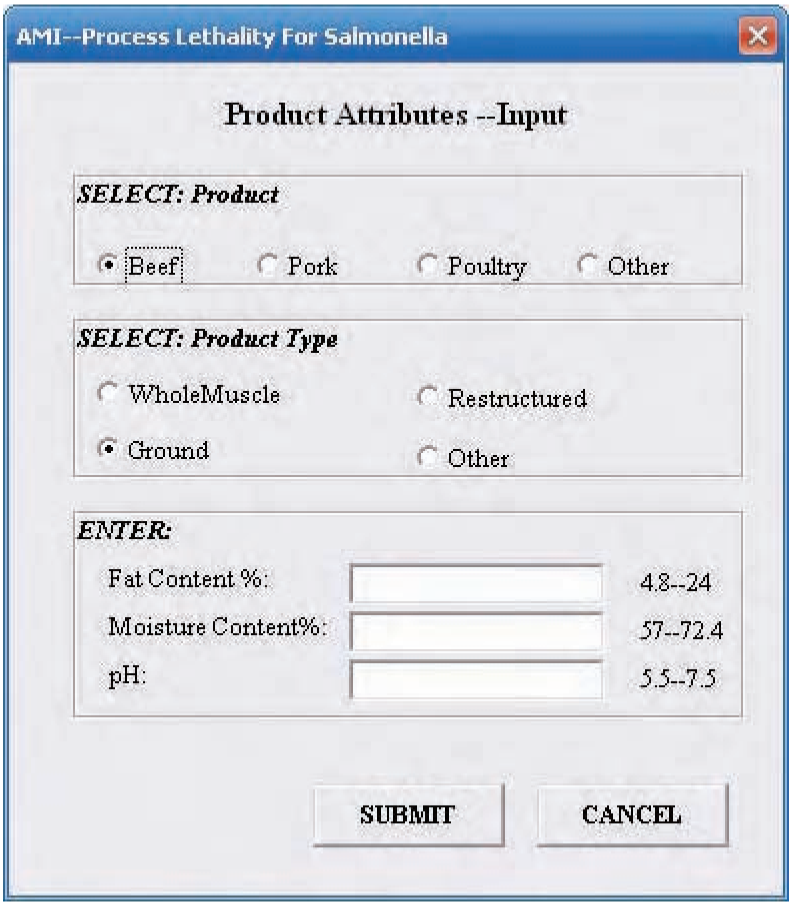

Figure 3. The front-end for input to the enhanced process lethality spreadsheet.

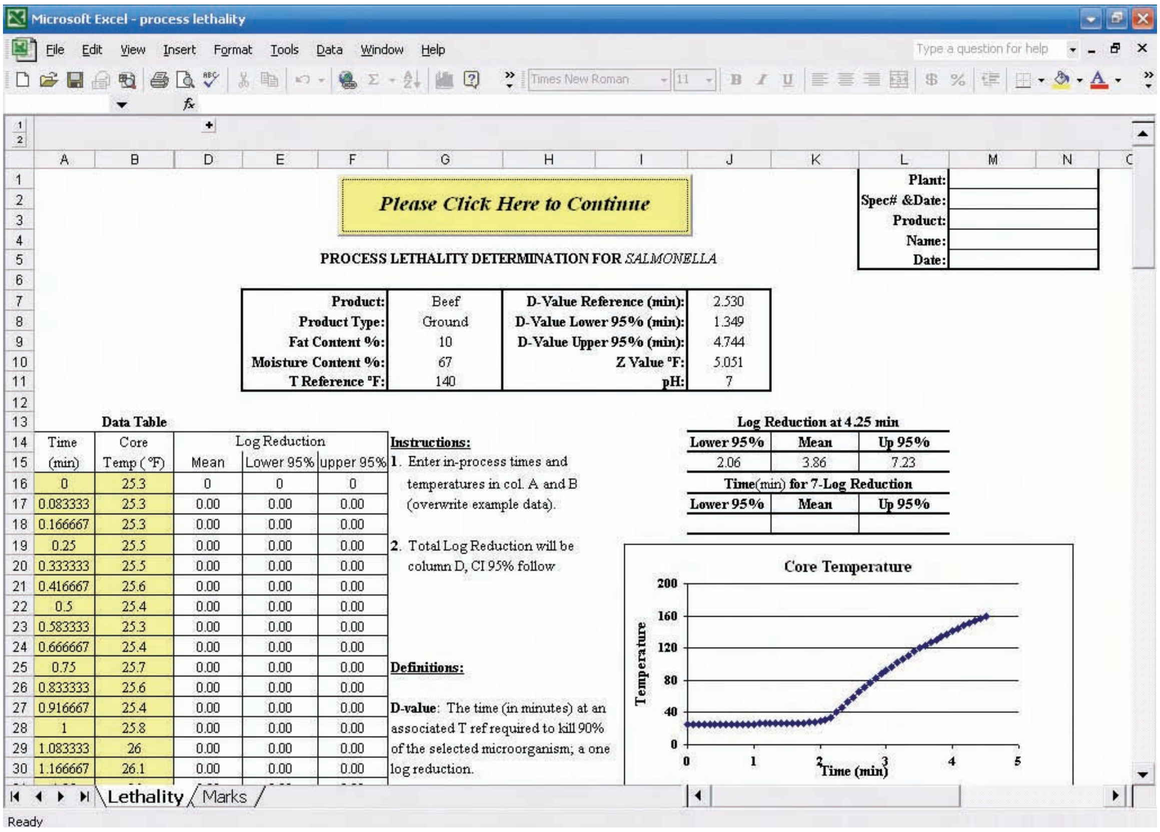

Figure 4. The spreadsheet showing product attributes, reference D-value with 95% confidence limit, and resulting log reductions with confidence limits.